7-2017

Antioxidant and anti-dyslipidemic effects of polysaccharidic extract from sea cucumber processing liquor

Hang Qi
National Engineering Research Center of Seafood

Xiaolin Ji
National Engineering Research Center of Seafood

Shan Liu
National Engineering Research Center of Seafood

Dingding Feng
National Engineering Research Center of Seafood

Xiufang Dong
National Engineering Research Center of Seafood

Antioxidant and anti-dyslipidemic effects of polysaccharidic extract from sea cucumber processing liquor

Abstract

Sea cucumber is a seafood of high nutritional value. During its processing, sea cucumber processing liquor is routinely produced, which is usually discarded as waste. The chemical composition of this processing liquor is similar to sea cucumbers themselves. Hence, valuable ingredients, such as functional polysaccharides, could be obtained from them.

Results

Biologically active polysaccharides from sea cucumber processing liquor were extracted through protease hydrolysis and electroosmosis. The analysis revealed that the polysaccharide extract from sea cucumber processing liquor (PESCPL) is predominantly composed of mannose, in addition to some glucose and fucose. The antioxidant activity of PESCPL was analyzed using *in vitro*. It was demonstrated that PESCPL could effectively scavenge 1,1-diphenyl-2-picrylhydrazyl radicals, hydroxyl radicals, and superoxide anion radicals. The effect of PESCPL was investigated *in vivo* by using mice model fed with high-fat diets with/without PESCPL supplement. It was shown that PESCPL could increase the catalase and superoxide dismutase activity in the serum and decrease serum malonaldehyde content. Furthermore, mice fed with PESCPL diet showed a considerable decrease in the serum cholesterol and triglyceride levels and an increase in high-density lipoprotein cholesterol levels.

Conclusions

Our research highlights that PESCPL is a natural antioxidant and could be utilized as a therapeutic supplement for dyslipidemia.

Keywords

Biologically active polysaccharides, Catalase, Cholesterol, Electroosmosis, Electron spin resonance, Glucose, Mannose, Seafood, Superoxide dismutase, TriglyceridesWaste

Disciplines

Agriculture | Bioresource and Agricultural Engineering

Comments

This article is published as Qi, Hang, Xiaolin Ji, Shan Liu, Dingding Feng, Xiufang Dong, Baoyu He, Janaswamy Srinivas, and Chenxu Yu. "Antioxidant and anti-dyslipidemic effects of polysaccharidic extract from sea cucumber processing liquor." *Electronic Journal of Biotechnology* 28 (2017). DOI: 10.1016/j.ejbt.2017.04.001. Posted with permission.

Authors

Hang Qi, Xiaolin Ji, Shan Liu, Dingding Feng, Xiufang Dong, Baoyu He, Janaswamy Srinivas, and Chenxu Yu

Electronic Journal of Biotechnology 28 (2017) 1–6

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Antioxidant and anti-dyslipidemic effects of polysaccharidic extract from sea cucumber processing liquor

Hang Qi [a,*], Xiaolin Ji [a], Shan Liu [a], Dingding Feng [a], Xiufang Dong [a], Baoyu He [a], Janaswamy Srinivas [b], Chenxu Yu [c]

[a] School of Food Science and Technology, Dalian Polytechnic University, National Engineering Research Center of Seafood, Dalian 116034, PR China
[b] Department of Dairy and Food Science, South Dakota State University, Brookings, SD57007 , USA
[c] Department of Agricultural and Biosystems Engineering, Iowa State University, IA 50011, USA

ARTICLE INFO

Article history:
Received 19 October 2016
Accepted 13 April 2017
Available online 21 April 2017

Keywords:
Biologically active polysaccharides
Catalase
Cholesterol
Electroosmosis
Electron spin resonance
Glucose
Mannose
Seafood
Superoxide dismutase
Triglycerides
Waste

ABSTRACT

Background: Sea cucumber is a seafood of high nutritional value. During its processing, sea cucumber processing liquor is routinely produced, which is usually discarded as waste. The chemical composition of this processing liquor is similar to sea cucumbers themselves. Hence, valuable ingredients, such as functional polysaccharides, could be obtained from them.
Results: Biologically active polysaccharides from sea cucumber processing liquor were extracted through protease hydrolysis and electroosmosis. The analysis revealed that the polysaccharide extract from sea cucumber processing liquor (PESCPL) is predominantly composed of mannose, in addition to some glucose and fucose. The antioxidant activity of PESCPL was analyzed using *in vitro*. It was demonstrated that PESCPL could effectively scavenge 1,1-diphenyl-2-picrylhydrazyl radicals, hydroxyl radicals, and superoxide anion radicals. The effect of PESCPL was investigated *in vivo* by using mice model fed with high-fat diets with/without PESCPL supplement. It was shown that PESCPL could increase the catalase and superoxide dismutase activity in the serum and decrease serum malonaldehyde content. Furthermore, mice fed with PESCPL diet showed a considerable decrease in the serum cholesterol and triglyceride levels and an increase in high-density lipoprotein cholesterol levels.
Conclusions: Our research highlights that PESCPL is a natural antioxidant and could be utilized as a therapeutic supplement for dyslipidemia.

1. Introduction

Hyperlipidemia and dyslipidemia are considered contributing factors for a number of health concerns, *e.g.*, atherosclerosis, obesity, heart disease, stroke, and kidney failure [1]. Elevated levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) coupled with low serum high-density lipoprotein cholesterol (HDL-C) levels are considered to be significant risk factors for atherosclerosis [2]. In addition, atherosclerosis is often accompanied by the production of free radicals by endothelial and vascular smooth muscles [3]. Excessive free radicals such as hydroxyl radicals and superoxide radicals are toxic and indeed result in organ damage and accelerate the aging process [4,5]. Enzymes with antioxidant activities such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase serve as scavengers of free radicals [6] to ameliorate their harmful effects.

The conventional therapeutic protocols for addressing hyperlipidemia and dyslipidemia are lipid-lowering drugs such as simvastatin, atorvastatin, lovastatin, and fibrates. Recently, considerable efforts are being devoted to obtain biologically active components from natural sources, and polysaccharides stand out as promising alternatives. Polysaccharides are natural materials and are available from widespread sources. Several of them display antioxidant and anti-dyslipidemic activities, *e.g.*, polysaccharides from *Pleurotus eryngii* [7], *Catathelasma ventricosum* [8], *Pholiota nameko* [9], and *Cordyceps militaris* [10]. Functional polysaccharides have also been extracted and studied from marine animal origins, such as sea cucumber [11,12,13], abalone [14], and abalone gonad [15]. However, a rich reservoir of functional polysaccharides from processing the by-products of these marine species remains to be fully explored.

* Corresponding author.
E-mail address: qihang@dlpu.edu.cn (H. Qi).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2017.04.001

Sea cucumbers are a precious resource of substances with medicinal importance. Their production reached more than 200 thousand tons in the year 2014, with a market value of around $30 million [16]. Chinese and other East/Southeast Asians consume the majority of them. Sea cucumbers contain more than 50 kinds of nutrients including amino acids, essential/polyunsaturated fatty acids, vitamins, and trace elements and active substances such as polysaccharides, collagen proteins, saponins, and brain glycosides [15]. However, they undergo strong autolysis soon after the animal is taken out of the seawater [17,18], especially under UV radiation, which leads to severe economic losses. To circumvent this quandary, fresh sea cucumbers are being processed into instant and/or semi-dried products [15]. This process involves several cycles of heating and cooking to deactivate the autolyzing enzymes. However, such a protocol results in large quantities (about 10 million tons annually) of processing liquor that is currently being discarded directly into the sea as a wasteful byproduct. The chemical composition of this processing liquor is similar to sea cucumbers themselves [19]. Hence, valuable ingredients, such as functional polysaccharides, could be obtained from them. In this study, we explored methods of extraction and characterization of polysaccharide extract from sea cucumber processing liquor (PESCPL) and characterized its antioxidant and anti-dyslipidemic activities to illustrate its potential applications as therapeutic supplements and/or functional foods.

2. Materials and methods

2.1. Materials and reagents

Sea cucumber processing liquor (SCPL) was collected from Dalian Bangchuidao Seafood Co. Ltd. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), trichloroacetic acid, and standards of monosaccharides and dextrans (molecular weight: 788, 404, 212, 112, 47.0, 11.8, and 5.9 kDa) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Assay kits for malonaldehyde (MDA), CAT, SOD, total cholesterol (TC), total TG, and HDL-C were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Other reagents used in this study were of the highest quality available from commercial vendors.

2.2. Animals

Male BALB/c strain rats were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Certificate No. SCXK.2012-0001, Beijing, China) and acclimatized for 1 week before experiment. Rodent laboratory chow and tap water were provided *ad libitum*, and mice were housed in animal cages at a temperature of 24 ± 1°C with a 12/12 h light/dark cycle and 50 ± 10% humidity. All the procedures were in strict accordance with People's Republic of China's legislations on the use and care of laboratory animals and guidelines established by the Institute for Experimental Animals of Shandong University and were approved by the University Committee for Animal experiments (authorization date is March 1, 2015; authorization number is AEC2015-024). During the feed stage, the average intake of each rat was 3.7 ± 0.5 g; this was calculated by animal keepers. Therefore, food intakes were not affected by body lipid metabolism.

2.3. Preparation of PESCPL

First, 95% ethanol was added to SCPL (same batches of concentrated liquor from more than 250 kg processing liquor) to a final 80% ethanol content. After leaving it overnight, the precipitate was collected by centrifugation at 2500 × g at 4°C for 20 min. The dried precipitate was lyophilized, and crude polysaccharides were extracted with acetone addition (1:3 w:v). The product was centrifuged at 2500 × g at 4°C for 10 min, and the supernatant was removed. This procedure was repeated three times, and the final residue was placed in a fume hood for 3 h to remove excess acetone. Subsequently, it was dissolved in distilled water with 1% neutral protease, and the pH of the solution was adjusted to 7.0. The solution was incubated at 55°C for 3 h before denaturing the enzyme at 100°C for 10 min. The pH of the solution was then adjusted to 8.0, and the solution was incubated at 37°C for 3 h with 1% trypsin. Subsequently, the trypsin and other proteins were denatured at 100°C for 10 min. After vigorous stirring, 80% ethanol was added to the solution and stored overnight. The precipitate was then collected by centrifugation (2500 × g at 4°C for 20 min), dissolved in water, and subjected to electrodialysis at 20 V for 1 h to remove the salt. Afterward, the solution was stored overnight with 80% ethanol. Finally, the precipitate was collected by centrifugation (2500 × g at 4°C for 10 min) and freeze–dried.

2.4. Compositional analysis

Moisture, ash, crude protein, lipid, and sugars contents were determined according to AOAC methods [20]. All experiments were performed in triplicate and presented as means ± standard deviations (n = 3).

2.5. Analysis of monosaccharide composition

Reversed-phase, high-performance liquid chromatography (HPLC) after pre-column derivatization and UV detection was used in this study for monosaccharide compositional analysis [21]. Briefly, 5 mg of PESCPL was hydrolyzed in 1 mL 2 mol/L trifluoroacetic acid solution at 130°C for 2 h. Excess acid was removed by co-distillation with the addition of 1 mL methanol. The dry hydrolysate was dissolved in 100 μL of 0.3 mol/L $NH_3 \cdot H_2O$, and 120 μL of 0.5 mol/L methanol solution of 1-phenyl-3-methyl-5-pyrazolone (PMP) was added at 70°C for 1 h. Later, 100 μL of 0.3 mol/L HCl was added; the mixture was vigorously vortexed and then centrifuged at 2400 × g for 5 min. The supernatant containing the labeled carbohydrate was filtered through 0.22 μm nylon membranes (MSI, Westborough, MA, USA), and 10 μL of the filtrate was injected into the C18 column. The mobile phase was a mixture of 0.1 mol/L KH_2PO_4 (pH 10) solution and acetonitrile (83:17). The flow rate was 1.0 mL/min and column temperature was 30°C. Compositional identification was done by comparing the LC spectrum of hydrolysate with the spectrum of reference standards (D-mannose, N-acetyl-D-glucosamine, D-glucuronic acid, D-galacturonic acid, N-acetyl-D-galactosamine, D-glucose, D-galactose, D-xylose, L-arabinose, and L-fucose).

2.6. Determination of scavenging activity for radicals

Hydroxyl radical scavenging activity was determined using the electron spin resonance (ESR) spectrometric methodology. The PESCPL samples were dissolved in deionized water to obtain a serial of concentrations of 31.25, 62.5, 125, 250, and 500 mg/mL. The sample (10 μL) was mixed with deionized water (52 μL), 6 mM EDTA-Na_2 (10 μL), 6% H_2O_2 (8 μL), and 1 M DMPO (10 μL). The reaction was initiated by adding 10 μL of 6 mM $FeSO_4$ to the mixture. The mixture was then incubated in water bath at 40°C for 30 min. Then, 40 μL of the sample was transferred into a glass capillary tube (Blaubrand intraMARK, Brand, Germany) at room temperature for spectral acquisition with an ESR spectrometer A200 (Bruker, Karisruhe, Germany). The specific conditions used were receiver gain: 1.0×10^5, modulation amplitude: 1.0 G, microwave power: 74.8 mw, microwave frequency: 9.44 GHz, time constant: 163.84 ms, conversion time: 480 ms and modulation frequency: 100.00 kHz. Deionized water was used as control. The amplitude of the second peak of the spectrum represents the amount of DMPO-OH adducts, which is related to the amount of hydroxyl radicals scavenged.

DPPH radical scavenging activity was measured using the ESR. A sample solution of 10 μL of PESCPL at different concentrations (10, 12.5, 15, 17.5, and 20 mg/mL, respectively) was added to 20 μL of 500 μM DPPH in methanol to a final volume of 50 μL with deionized water. After mixing vigorously, the solution was incubated for 30 min in dark at room temperature. Subsequently, it was transferred into a glass capillary tube (Blaubrand intraMARK, Brand, Germany) for spectral acquisition at room temperature using an ESR spectrometer, A200 (Bruker, Karisruhe, Germany). The experimental conditions were 1.42 $\times$ 10^4 of receiver gain, 1.0 G of modulation amplitude, 5.32 mw of microwave power, 9.44 GHz of microwave frequency, 81.82 ms of time constant, 40 ms of conversion time, and 100.00 kHz of modulation frequency.

Superoxide radical scavenging activity was determined using the ESR method described by Qi et al. [22] with a little modification. In brief, the typical incubation mixture was composed of 5 μL hypoxanthine (HPx, 500 μM), 1 μL xanthine oxidase (XOD, 0.7 unit/mL), 5 μL DETAPAC, 7.5 μL DMPO (1 M), 21.5 μL phosphate buffer (50 mM at pH 7.4), and 10 μL PESCPL. After mixing vigorously for 10 s, the solution was transferred into a glass capillary tube (Blaubrand intraMARK, Brand, Germany), and ESR spectra were recorded at room temperature using an ESR spectrometer A200 (Bruker, Karisruhe, Germany). The spectrum was recorded by the software provided with the instrument using 5.02 $\times$ 10^5 receiver gain, 1.0 G modulation amplitude, 1.17 mw microwave power, 9.438 GHz microwave frequency, 2621.44 ms time constant, 480 ms conversion time, and 100.00 kHz modulation frequency.

2.7. Assays of MDA, SOD, and CAT activities

Rats were anesthetized and sacrificed by cervical decapitation. Blood samples were collected and centrifuged for 10 min at 1000 $\times$ g to obtain the serum. Serum homogenates were used for assays of MDA, SOD, and CAT activities using appropriate activity assay kits.

2.8. Anti-dyslipidemic effect of PESCPL in rats

Male BALB/c rats aged 28 days were used in this study. The composition of the high-fat diet was as follows: 78.8% basal feed, 1% cholesterol, 0.2% sodium cholate, 10% custard powder, and 10% lard oil. The energy content of the diet is 444.9 kcal/100 g. Ninety rats were fed with a basic diet for 7 days to acclimatize to animal facilities. They were then weighed and randomly divided into six groups of 15 animals each. Two different protocols were adopted in this study. In all experiments, rules governing the use and care of laboratory animals set by Chinese legislations and the University Committee for animal experiments were strictly observed.

Protocol I: The first group (control group) was fed with the common commercial rat chow, while the second group, as dyslipidemic control, received the high-fat diet. The third, fourth, and fifth groups received the high-fat diet with PESCPL supplemented at dosages of 400, 100, and 25 mg/kg, respectively. The sixth group (positive control) received the high-fat diet with the administration of an anti-dyslipidemia drug, simvastatin, at a dosage of 20 mg/kg. The animals were treated for 30 days and had *ad libitum* access to food and water.

Protocol II: In each group, 15 rats were used. The mice were first fed with the high-fat diet for 20 days, and their TC levels were measured on the 21st day. Once the rats exhibited dyslipidemic symptoms, they were treated and tested with the procedure described in protocol I.

At the end of each experiment, the rats were fasted for 18 h. Blood was then collected from the tail and centrifuged to obtain the serum for late lipid profile analysis. Serum TC, TG, and HDL-C levels were determined with commercially available kits.

Table 1
Chemical composition of PESCPL.

Component	PESCPL (%)
Moisture	2.81 ± 0.34
Ash	12.25 ± 1.28
Lipid	1.19 ± 0.64
Crude Protein	14.06 ± 2.31
Carbohydrate	69.69 ± 4.52

2.9. Statistical analysis

Results were presented as mean ± standard deviation. One-way analysis of variance was used for data analysis using SPSS 16.0. The significance was set at $P < 0.05$ and $P < 0.01$ levels.

3. Results

3.1. Chemical analysis

In this study, an improved method of hydrolysis by combining neutral protease and trypsin coupled with electroosmosis treatment was developed to prepare the PESCPL. The chemical composition of the PESCPL is listed in Table 1, which indicates that it contains 69.69% carbohydrate, 14.06% protein, 2.81% water, 12.25% ash, and 1.19% lipid. The monosaccharide composition analysis (Fig. 1) revealed that mannose, glucose, and fucose are the main constituents of the carbohydrate components of the PESCPL, although the content of mannose is by far the highest.

3.2. Antioxidant activity of PESCPL in vitro

To confirm that PESCPL has antioxidant functions, the scavenging ability of PESCPL on DPPH free radicals, hydroxyl radicals, and superoxide radicals was evaluated *in vitro* with biochemical tests. As shown in Fig. 2, PESCPL exhibits a high radical-scavenging activity, which positively correlates to the dosage. PESCPL's DPPH-scavenging ability reached 65.80 ± 0.58% at the concentration of 4 mg/mL (Fig. 2a). For hydroxyl radical, the highest scavenging ability was 55.00 ± 1.02% at a concentration of 50 mg/mL (Fig. 2b), apparently lower than its DPPH-scavenging ability. For superoxide anion radicals, it was 55.32 ± 0.50% at a concentration of 100 mg/mL (Fig. 2c), which was the lowest among the three radicals. Overall, these data suggest that the PESCPL could be effective as an antioxidant additive, but its effectiveness varies depending on the radicals.

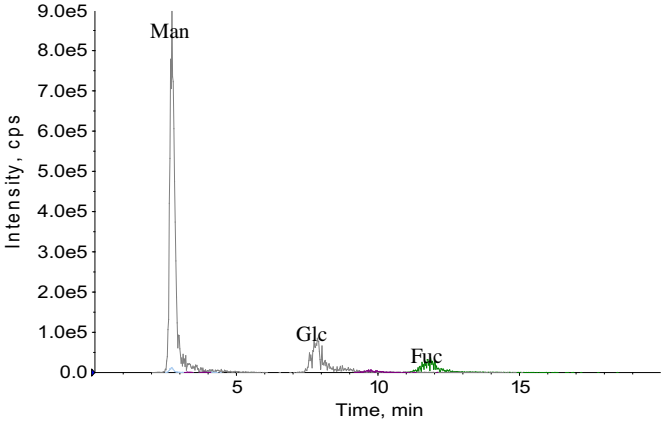

Fig. 1. HPLC analysis of monosaccharide composition of PESCPL (Man, mannose; Glc, glucose; Fuc, fucose).

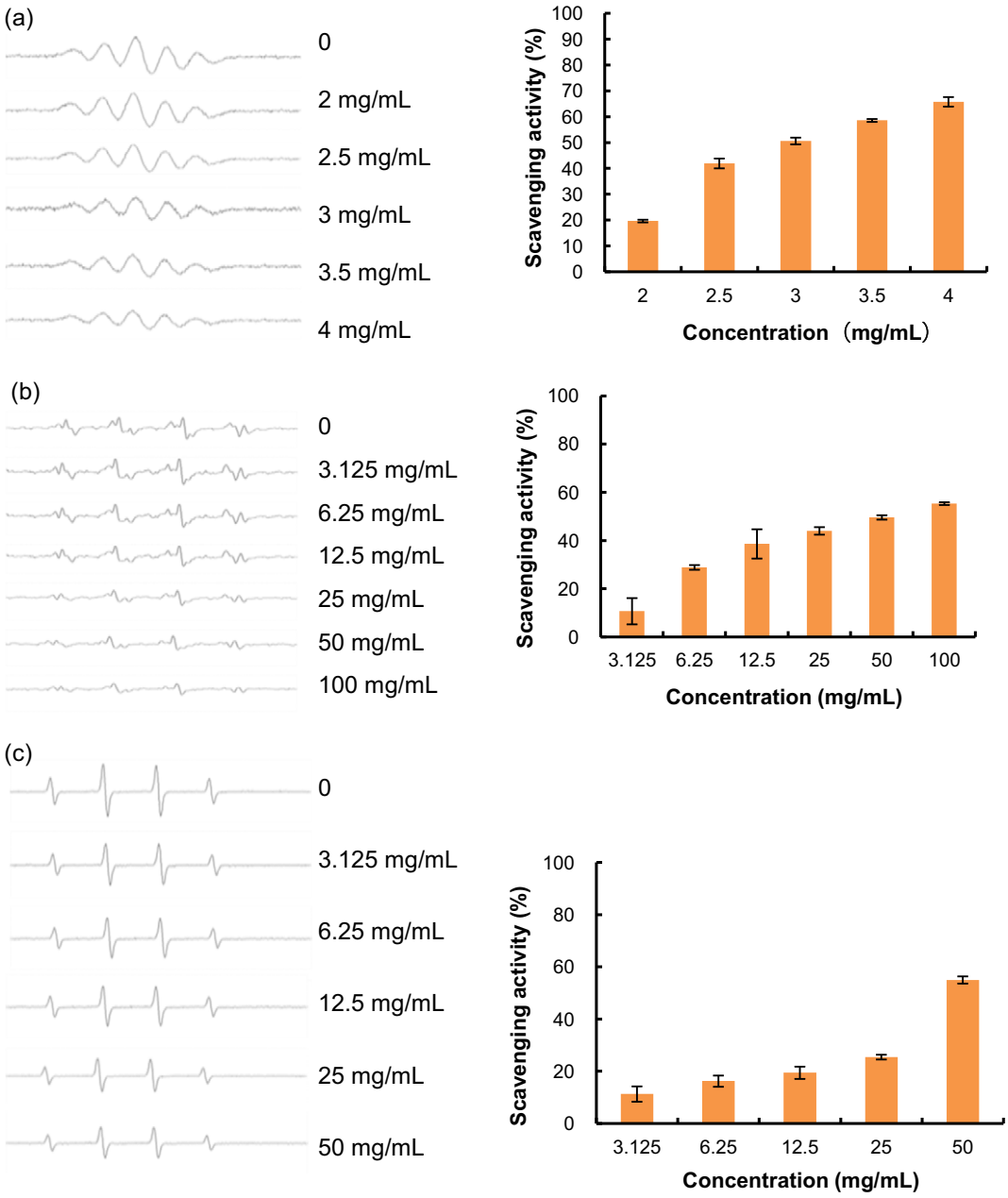

Fig. 2. Free radical scavenging of PESCPL. (a) DPPH scavenging ability; (b) superoxide radical scavenging ability; (c) hydroxyl radical scavenging ability.

3.3. Antioxidant activity of PESCPL in vivo

In order to assess the antioxidant functions of PESCPL *in vivo*, the SOD, MDA, and CAT levels in rats' blood were analyzed. As shown in Table 2 and Table 3, compared with the normal diet, feeding for 30 days with high-fat diet resulted in a decrease in CAT and SOD levels by about 50% (Table 2 and Table 3). However, in the groups that were fed the PESCPL to supplement the high-fat diet, CAT levels increased to 11.906 ± 4.335 and 10.545 ± 2.598 U/mL when fed 400 mg/kg PESCPL in protocols I and II, respectively. Similarly, the SOD levels also increased to 143.244 ± 18.979 and 148.530 ± 24.314 U/mL, respectively. In comparison, the MDA levels did not show significant difference across all samples regardless of the diet. The levels of CAT, SOD, and MDA nearly recovered in the normal group at the concentration of 400 mg/kg in both the protocols. Overall, the *in vivo* results clearly indicate that PESCPL is effective in increasing the activities of antioxidant enzymes and removing the free radicals.

Table 2
Effect of PESCPL on serum level of antioxidant indices in rats fed a high-fat diet in protocol I (x ± s, n = 15).

Group	Dose (mg/kg)	CAT (U/ml)	SOD (U/ml)	MDA (nmol/ml)
Normal	–	9.774 ± 1.355	149.057 ± 24.828	11.763 ± 3.597
Control	–	4.776 ± 1.879$^{\triangle\triangle}$	70.333 ± 27.583$^{\triangle\triangle}$	15.118 ± 3.347$^{\triangle\triangle}$
P400	400	11.906 ± 4.335**	143.244 ± 18.979**	11.643 ± 2.127**
P100	100	7.473 ± 1.758**	123.782 ± 28.992**	11.552 ± 3.050**
P25	25	5.613 ± 2.190	91.864 ± 21.757*	14.161 ± 2.919
Sim20	20	6.612 ± 1.503*	121.284 ± 23.465**	11.347 ± 2.753**

$^{\triangle\triangle}$ $P < 0.01$, compared with normal Group.
** $P < 0.01$, compared with the Control Group.
* $P < 0.05$, compared with the Control Group.

Table 3
Effect of PESCPL on serum level of antioxidant indices in rats fed a high-fat diet in protocol II (x ± s, n = 15).

Group	Dose (mg/kg)	CAT (U/ml)	SOD (U/ml)	MDA (nmol/ml)
Normal	–	10.563 ± 2.869	149.990 ± 12.455	12.167 ± 2.161
Control	–	5.257 ± 1.503$^{\Delta\Delta *}$	103.878 ± 25.201$^{\Delta\Delta}$	15.753 ± 4.487$^{\Delta\Delta}$
P400	400	10.545 ± 2.598**	148.530 ± 24.314**	9.574 ± 1.477**
P100	100	8.010 ± 2.510**	142.353 ± 22.754**	12.425 ± 2.615**
P25	25	5.721 ± 2.301	126.737 ± 21.014**	13.775 ± 3.555
Sim20	20	10.105 ± 2.323**	142.966 ± 20.007**	11.459 ± 3.120**

$^{\Delta\Delta}$ $P < 0.01$, compared with the Normal Group.
* $P < 0.05$, compared with Control Group.
** $P < 0.01$, compared with Control Group.

Table 4
Effect of PESCPL on body weight in rats fed a high-fat diet in protocol I (x ± s, n = 15).

Group	Dose (mg/kg)	Body weight (g)				
		initial	1 week	2 week	3 week	4 week
Normal	–	18.4 ± 0.8	19.6 ± 0.7	19.8 ± 0.9	21.5 ± 0.7	22.1 ± 1.1
Control	–	18.7 ± 0.8	20.1 ± 1.0	20.1 ± 1.1	21.6 ± 1.4	22.4 ± 1.7
P400	400	18.7 ± 0.7	20.0 ± 0.9	20.3 ± 1.3	21.5 ± 1.0	22.2 ± 1.1
P100	100	18.3 ± 1.0	19.9 ± 1.1	19.9 ± 1.4	21.4 ± 1.0	21.7 ± 1.0
P25	25	18.4 ± 0.6	20.1 ± 0.7	19.7 ± 1.1	21.2 ± 0.7	21.5 ± 0.6
Sim20	20	18.3 ± 0.6	20.0 ± 1.0	20.4 ± 1.0	21.4 ± 1.0	22.0 ± 1.4

3.4. Evaluation of anti-dyslipidemic activity of the PESCPL in vivo

As shown in Table 4 and Table 5, all rats started with similar initial body weights at 18.6 ± 0.6 g. The increase in body weights of rats fed with high-fat diets w/o PESCPL supplement showed no significant differences ($P > 0.05$).

Table 6 shows the effect of diet w/o the PESCPL on serum lipid levels. First, the serums levels of TC and TG in rats of the control group (*i.e.* fed with normal diet) were significantly lower ($P < 0.01$) than those of high-fat diet groups, indicating that high-fat food could effectively induce dyslipidemia in rats. However, supplementing the high-fat diet with PESCPL at 100 and 400 mg/kg remarkably reduced the TC and TG levels in the serum ($P < 0.01$) to 4.289 ± 0.393 and 1.378 ± 0.208 mmol/L, respectively, and increased the serum HDL-C levels to 1.699 ± 0.339 mmol/L. Thus, it appears that PESCPL could effectively prevent dyslipidemia ($P < 0.01$). Furthermore, in protocol II, after rats were fed with the high-fat diet, the serum TC and TG levels were significantly greater (Table 7) than those in the normal group ($P < 0.01$), reflecting that dyslipidemia had been induced by the high-fat diet but with no detectable difference between the groups. Interestingly, PESCPL administration has substantially reduced the serum levels of TC and TG. However, HDL-C levels increased (Table 7). The effects of PESCPL to lower blood lipid levels in dyslipidemic rats are comparable to those of the drug simvastatin.

Table 6
Effect of PESCPL on serum level of dyslipidemic indices in rats fed a high-fat diet in protocol I (x ± s, n = 15).

Group	Dose(mg/kg)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)
Normal	–	3.003 ± 0.500	1.522 ± 0.265	1.695 ± 0.352
Control	–	5.078 ± 0.399$^{\Delta\Delta}$	2.176 ± 0.415$^{\Delta\Delta}$	1.285 ± 0.302$^{\Delta\Delta}$
P400	400	4.289 ± 0.393**	1.378 ± 0.208**	1.699 ± 0.339**
P100	100	4.597 ± 0.352**	1.435 ± 0.419**	1.541 ± 0.205**
P25	25	4.884 ± 0.497	1.567 ± 0.327**	1.459 ± 0.510
Sim20	20	4.570 ± 0.577**	1.382 ± 0.394**	1.812 ± 0.548**

$^{\Delta\Delta}$ $P < 0.01$, compared with Normal Group.
** $P < 0.01$, compared with the Control Group.

4. Discussion

In recent years, increasing interest in human health, nutrition, and disease prevention has greatly boosted consumer's demand for functional foods. Although sea cucumbers have become attractive functional foods and sources of bioactive compounds [17,18], their value remains to be fully explored because valuable ingredients (*e.g.* sulfated polysaccharide) in the SCPL are being discarded as waste in the processing industry. Further processing of the SCPL to extract these polysaccharides can not only reduce the negative environmental impact but also generate added value in the form of functional PESCPL. Our data indicated that the content of PESCPL, which was prepared by our method, was quite high. The monosaccharide composition of PESCPL was mainly mannose, glucose, and fucose. The content of mannose was the most, which is much higher than the others.

It has been shown in this study that PESCPL can effectively scavenge DPPH, hydroxyl radicals, and superoxide anion radicals in a dose-dependent manner. When the rats was fed with high-fat diet, it was found that although PESCPL did not change the mice's weight, it could significantly increase serum CAT and SOD activity ($P < 0.01$) and decrease MDA content ($P < 0.01$). PESCPL possessed strong antioxidant activity both *in vitro* and *in vivo*. The antioxidant performance of the PESCPL was comparable to that of water-soluble the polysaccharidic extract of *P. eryngii* [7], polysaccharide from *L. japonica* [23], and polysaccharides from *C. ventricosum* [8].

Notably, not only had PESCPL shown antioxidant activity capabilities but also oral administration of PESCPL significantly lowered the levels of TC and TG and increased the HDL-C content ($P < 0.01$) in dyslipidemic mice in two different protocol models. In protocol I, oral administration of PESCPL to the rats on high-fat diets clearly prevented the occurrence of dyslipidemia and kept their serum lipid levels normal compared with those mice were fed a high-fat diet only. Moreover, in protocol II, when PESCPL was orally administered to mice that had been fed the high-fat diet and demonstrated dyslipidemia, PESCPL still could remarkably reduce the serum levels of TC and TG and increase HDL-C content. More notably, our study indicated that the PESCPL also showed strong *in vivo* anti-dyslipidemia effects. The PESCPL could act not only as a functional food additive to prevent dyslipidemia but also

Table 5
Effect of PESCPL on body weight in rats fed a high-fat diet in protocol II (x ± s, n = 15).

Group	Dose (mg/kg)	Body weight (g)					
		Pre-chemical treatment	Post-chemical treatment	1 week	2 week	3 week	4 week
Normal	–	18.2 ± 0.7	22.1 ± 1.4	23.0 ± 1.7	23.0 ± 1.6	23.2 ± 1.5	24.1 ± 1.7
Control	–	18.6 ± 0.8	22.1 ± 1.3	22.9 ± 1.5	23.0 ± 1.6	22.8 ± 1.3	23.7 ± 1.2
P400	400	18.6 ± 0.7	21.8 ± 0.8	23.0 ± 1.1	23.5 ± 0.9	23.6 ± 1.3	24.2 ± 1.3
P100	100	18.4 ± 0.8	22.2 ± 0.1	23.0 ± 1.2	23.2 ± 1.4	23.0 ± 1.4	23.8 ± 1.0
P25	25	18.4 ± 1.0	22.8 ± 1.1	23.5 ± 1.1	23.4 ± 1.2	23.9 ± 1.7	24.0 ± 1.5
Sim20	20	18.4 ± 0.6	21.8 ± 0.9	22.2 ± 0.8	23.0 ± 1.7	23.3 ± 0.7	24.2 ± 1.3

Table 7
Effect of PESCPL on serum level of dyslipidemic indices in rats fed a high-fat diet in protocol II (x ± s, n = 15).

Group	Dose(mg/kg)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)
Normal	–	3.528 ± 0.287	1.784 ± 0.184	1.871 ± 0.462
Control	–	5.031 ± 0.632$^{\triangle\triangle}$	2.020 ± 0.491$^{\triangle}$	1.106 ± 0.244$^{\triangle\triangle}$
P400	400	4.057 ± 0.359**	1.736 ± 0.205*	1.888 ± 0.306**
P100	100	4.546 ± 0.606**	1.666 ± 0.327**	1.641 ± 0.280**
P25	25	4.685 ± 0.374*	1.729 ± 0.215*	1.361 ± 0.400*
Sim20	20	4.100 ± 0.429**	1.595 ± 0.308**	1.647 ± 0.369**

$^{\triangle}$ P 0.05, compared with Normal Group.
$^{\triangle\triangle}$ $P < 0.01$, compared with Normal Group.
* $P < 0.05$, compared with the Control Group.
** $P < 0.01$, compared with the Control Group.

as a therapeutic agent to ameliorate symptoms of dyslipidemia. This raises the question of finding the underlying mechanisms behind the anti-dyslipidemic effects of the PESCPL in rats. One possible explanation is the PESCPL molecules bind with lipids and act as carriers to participate in the metabolism of cholesterol to accelerate the transport and excretion of serum lipids [10]. To pinpoint which components of the PESCPL might bind to lipids, more characterization assays for the components of the PESCPL are needed. Some preliminary work with HPLC indicated that the content of proteins was much lower than that of polysaccharides. It is highly unlikely that proteins are the main functional component of the PESCPL. The polysaccharide component of the PESCPL is believed to be the functional one. More studies are necessary to further characterize these bioactive components and their structures to confirm their interaction with lipids.

It is also possible that the anti-dyslipidemic function of the PESCPL is realized through the bile acid sequestration mechanism [24]. PESCPL can act as a stimulator of bile acid synthesis. Most bile acids are reabsorbed in the small intestine, and then returned to the liver so that the bile acid pool remains constant. Bile acid sequestering agents in the small intestine may interrupt the enterohepatic circulation by increasing the fecal excretion of bile acids to reduce bile acid returning to the liver. To compensate the loss of bile acid, the liver may increase its synthesis of bile acids by oxidation of more hepatic cholesterol, the only precursor to bile acids, thereby resulting in the decrease in the total blood cholesterol level [24].

5. Conclusion

PESCPL could significantly scavenge DPPH radicals, hydroxyl radicals, and superoxide anion radicals. *In vivo* assay showed that they can also increase serum CAT and SOD activities while decrease MDA content. Furthermore, PESCPL can also reduce TC and TG levels significantly, and increase HDL-C significantly in dyslipidemic rats. PESCPL are natural antioxidants that can be effective both preventively and therapeutically against dyslipidemia.

Financial support

This work was financially supported by The National High Technology Research and Development Program of China (863 Program) (No. 2014AA093602); Public science and technology research funds projects of ocean (No. 201505030-5); Program for Liaoning Excellent Talents in University (LNET, No. LR2015004); and Program for Dalian High-level Innovation Talents (2016RQ063).

References

[1] Brown WV. Lipoprotein disorders in diabetes mellitus. Med Clin North Am 1994;78:143–61. https://doi.org/10.1016/S0025-7125(16)30180-8.
[2] Elahi MM, Kong YX, Matata BM. Oxidative stress as a mediator of cardiovascular disease. Oxid Med Cell Longev 2009(2):259–69. http://dx.doi.org/10.4161/oxim.2.5.9441.
[3] Sobey CG, Judkins CP, Rivera J, Lewis CV, Diep H, Lee HW, et al. NOX1 deficiency in apolipoprotein E-knockout mice is associated with elevated plasma lipids and enhanced atherosclerosis. Free Radic Res 2015;49:186–98. http://10.3109/10715762.2014.992893.
[4] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44. http://dx.doi.org/10.1016/j.biocel.2006.07.001.
[5] Vaz JA, Barros L, Martins A, Santos-Buelga C, Vasconcelos MH, Ferreira ICFR. Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. Food Chem 2011;126:610–6. http://dx.doi.org/10.1016/j.foodchem.2010.11.063.
[6] Hong YK, Wu HT, Ma T, Liu WJ, He XJ. Effects of *Glycyrrhiza glabra* polysaccharides on immune and antioxidant activities in high-fat mice. Int J Biol Macromol 2009;45:61–4. http://dx.doi.org/10.1016/j.ijbiomac.2009.04.001.
[7] Chen JJ, Mao D, Yong YY, Li JL, Wei H, Lu L. Hepatoprotective and hypolipidemic effects of water-soluble polysaccharidic extract of *Pleurotus eryngii*. Food Chem 2012;130:687–94. http://dx.doi.org/10.1016/j.foodchem.2011.07.110.
[8] Liu YT, Sun J, Rao SQ, Su YJ, Yang YJ. Antihyperglycemic, antihyperlipidemic and antioxidant activities of polysaccharides from *Catathelasma ventricosum* in streptozotocin-induced diabetic mice. Food Chem Toxicol 2013;57:39–45. http://dx.doi.org/10.1016/j.fct.2013.03.001.
[9] Zheng L, Zhai GY, Zhang JJ, Wang LQ, Ma Z, Jia MS, et al. Hyperlipidemic and hepatoprotective activities of mycelia zinc polysaccharide from *Pholiota nameko* SW-02. Int J Biol Macromol 2014;70:523–9. http://dx.doi.org/10.1016/j.ijbiomac.2014.07.037.
[10] Wang LQ, Xu N, Zhang JJ, Zhao HJ, Lin L, Jia SH, et al. Antihyperlipidemic and hepatoprotective activities of residue polysaccharide from *Cordyceps militaris* SU-12. Carbohydr Polym 2015;131:355–62. http://dx.doi.org/10.1016/j.carbpol.2015.06.016.
[11] Liu HH, Ko WC, Hu ML. Hypolipidemic effect of glycosaminoglycans from the sea cucumber *Metriatyla scabra* in rats fed a cholesterol-supplemented diet. J Agric Food Chem 2002;50:3602–6. http://dx.doi.org/10.1021/jf020070k.
[12] Diao HX, Song SL, Liang H, Wang YS, Wang WL, Ji AG. Protective effect of polysaccharides from sea cucumber on glu-induced neurotoxicity in PC12 cells. Zhong Yao Cai 2009;32:398–400.
[13] Liu X, Sun ZL, Zhang MS, Meng XM, Xia XK, Yuan WP, et al. Antioxidant and antihyperlipidemic activities of polysaccharides from sea cucumber *Apostichopus japonicus*. Carbohydr Polym 2012;90:1664–70. http://dx.doi.org/10.1016/j.carbpol.2012.07.047.
[14] Zhu BW, Zhao JG, Yang JF, Tada M, Zhang ZS, Zhou DY. Purification and partial characterization of a novel β-1,3-glucanase from the gut of sea cucumber *Stichopus japonicus*. Process Biochem 2008;43:1102–6. http://dx.doi.org/10.1016/j.procbio.2008.06.001.
[15] Zhu BW, Zhou DY, Li T, Yan S, Yang JF, Li DM, et al. Chemical composition and free radical scavenging activities of a sulphated polysaccharide extracted from abalone gonad (*Haliotis Discus Hannai* Ino). Food Chem 2010;121:712–8. http://dx.doi.org/10.1016/j.foodchem.2010.01.010.
[16] Ministry of Agriculture Fishery Bureau. China fishery statistical yearbook 2015. Beijing: China Agricultural Publishing House; 2015.
[17] Qi H, Dong XF, Zhao YP, Li N, Fu H, Feng DD, et al. Ros production in homogenate from the body wall of sea cucumber *Stichopus japonicus*, under UVA irradiation: ESR spin-trapping study. Food Chem 2016;192:358–62. http://dx.doi.org/10.1016/j.foodchem.2015.07.030.
[18] Qi H, Fu H, Dong XF, Feng DD, Nan L, Wen CR, et al. Apoptosis induction is involved in UVA-induced autolysis in sea cucumber *Stichopus japonicus*. J Photochem Photobiol B 2016;158:130–5. http://dx.doi.org/10.1016/j.jphotobiol.2016.02.034.
[19] Chen D, Yang XC, Cao WJ, Guo YX, Sun YQ, Xiu ZL. Three-liquid-phase salting-out extraction of effective components from waste liquor of processing sea cucumber. Food Bioprod Process 2015;96:99–105. http://dx.doi.org/10.1016/j.fbp.2015.07.002.
[20] AOAC. Official methods of analysis of the association of official analytical chemists. 15th ed. Washington: Association of Official Analytical Chemists; 1990.
[21] Chen RZ, Liu ZQ, Zhao JM, Chen RP, Meng FL, Zhang M, et al. Antioxidant and immunobiological activity of water-soluble polysaccharide fractions purified from acanthopanax senticosu. Food Chem 2011;127:434–40. http://dx.doi.org/10.1016/j.foodchem.2010.12.143.
[22] Qi H, Kawagishi M, Yoshimoto M, Takano H, Zhu BW, Shimoishi Y, et al. Artificial food colorants inhibit superoxide production in differentiated hl-60 cells. Biosci Biotechnol Biochem 2010;74:1725–8. http://dx.doi.org/10.1271/bbb.100314.
[23] Zha XQ, Xiao JJ, Zhang HN, Wang JH, Pan LH, Yang XF, et al. Polysaccharides in *Laminaria japonica* (LP): Extraction, physicochemical properties and their hypolipidemic activities in diet-induced mouse model of atherosclerosis. Food Chem 2012;134:244–52. http://dx.doi.org/10.1016/j.foodchem.2012.02.129.
[24] Qi HM, Huang LY, Liu XL, Liu DM, Zhang QB, Liu SM. Antihyperlipidemic activity of high sulfate content derivative of polysaccharide extracted from *Ulva pertusa* (Chlorophyta). Carbohydr Polym 2012;87:1637–40. http://dx.doi.org/10.1016/j.carbpol.2011.09.073.